HLA-D: The T Cell Perspective

Fritz H. Bach, Nobuo Onta, Andrea Anichini, and Nancy L. Reinsmoen

Immunobiology Research Center

Departments of Laboratory

Medicine/Pathology and Surgery

University of Minnesota

1985

This is paper #420 from the Immunobiology Research Center, University of Minnesota, Box 724 Mayo, Minneapolis, MN 55455. This work was supported in part by NIH grants AI 17687, AI 18326, AM 13083, AI 19007, ONR contract N00014-85-K-0004 ~~N00014-85-K-004~~, March of Dimes grant 6-425, and National Multiple Sclerosis Society grant RG 1505-A-1, and the Harry Kay Charitable Foundation.

ABBREVIATIONS

HTC homozygous typing cell

MLC mixed leukocyte culture

PLT primed lymphocyte typing

IEF isoelectric focusing

Cellular Detection of HLA Class II Encoded Determinants--
Subtype Polymorphisms of HLA-D

INTRODUCTION

The introduction of the mixed leukocyte culture test (MLC) (Bain et al. 1964; Bach and Hirschhorn 1964) combined with the establishment of a one-way method of stimulation in MLC (Bach and Voynow 1966) allowed the correlation of proliferative response in MLC with results of serological studies and the suggestion that reactivity in MLC is controlled by the major histocompatibility complex in man (Bach and Amos 1967), now known as HLA. That the chromosomal region of HLA which controls the strongest stimulatory determinants for MLC (now known as the HLA-D region) was different from the class I encoding genes was already suggested in two early studies, (Amos and Bach 1968; Bach et al. 1969); definitive evidence in this regard came from the studies of Yunis and Amos (1971).

A series of technical and conceptual advances led to our present day understanding of the D region. Homozygous typing cells (HTCs) (Mempel et al. 1973; van den Tweel et al. 1973; Jorgensen et al. 1973; Dupont et al. 1973) were used in MLC, which allowed the definition of Dw "specificities," based on the demonstration that response in MLC can be meaningfully quantitated with regard to genetic disparity (Albertini and Bach 1968). In the same year, serological recognition of HLA-D region encoded antigens was accomplished by van Leeuwen, van Rood and co-workers (1973).

In 1975, the primed LD (lymphocyte) typing (PLT) test was described from our laboratories (Sheehy et al. 1975) in an attempt to help define T lymphocyte recognized specificities for which no HTC existed, as well as to obtain a finer analysis of such T lymphocyte recognized determinants. A further refinement of that test involved the cloning of T cells responsive in

MLC/PLT in mouse (Fathman and Hengarten 1978) and in man (Bach et al. 1979).

Use of HTCs led to recognition that more than a single Dw specificity could be associated with a serologically defined antigen such as DR4. These Dw specificities were subtypic to the serologically defined antigen, i.e. DR4 could be "split" into a number of Dw specificities. With the recognition that the serologically defined antigens of DR and DQ can be divided into Dw subtypes with HTC testing, it became important to evaluate whether such Dw subtype polymorphisms can be related to DR and DQ protein polymorphisms. Although DR β chain polymorphism was demonstrated within a single serologically defined DR antigen (e.g. Goyert and Silver 1982), we were able to demonstrate that a highly significant correlation existed between the Dw subtype of the cell from which the DR β proteins were taken and the positional variation of those proteins in isoelectric focusing (Groner et al. 1983), a finding independently confirmed by Nepom et al. (1983). These findings support the concept that the Dw subtypes were related to protein differences.

It is our purpose in this paper to discuss our current knowledge of the HLA-D region from the T cell perspective, an area that has been recently reviewed (Bach 1985). A brief introduction to the HLA-D region as it is known today may be useful in this regard.

THE GENETIC ORGANIZATION OF HLA

Within the HLA-D region, there are three different families of genes called DR, DQ, and DP. Within the DR family, there are three β genes and a single α gene, the product of which associates with the products of DR β genes. The DR α gene is essentially invariant but the β genes are polymorphic to an extent which will be discussed below. The DQ family includes two α genes, $DQ\alpha_1$ and $DQ\alpha_2$ and

two β genes, $DQ\beta_1$ and $DQ\beta_2$. The DP family also consists of two α and two β genes, $DP\alpha_1$, $DP\alpha_2$, $DP\beta_1$ and $DP\beta_2$. In addition to the DR, DQ and DP α and β genes, there is an additional α chain gene, referred to as DZα, the map position of which is not known and which does not appear to fit into any one of the three families, as well as recent, and as yet unpublished, data (E. Long, personal communication), suggesting the existence of an additional β gene.

Limited data that are available are consistent with the model that (in many cases) the DR Ia specificities (DR1 through DRw14) are present on β chains encoded by allelic genes. With this model one can, therefore, by convention refer to the $DR\beta_1$ (or $\alpha\beta_1$) genes, and their products as carrying these serologically defined specificities. Two serologically defined specificites, DRw52 and 53 (formerly known as MT2 and MT3) appear to be associated, in some cases, with a different DR αβ dimer that could be referred to as $DR\alpha\beta_2$ (Hurley et al. 1984). Additional work will be needed before these associations with $\alpha\beta_1$ and $\alpha\beta_2$ can be generalized to the extent just discussed; it is quite possible, if not likely, that there will be exceptions to this rule. We shall, however, use this model to discuss the data to be presented recognizing the caveats that must be considered.

The DQw1 (equivalent to MT1 and MB1), DQw2 (MB2) and DQw3 (MB3) specificities are associated with the expression of DQ αβ dimers; once again, to the extent that genes encoding these specificities are allelic, they could arbitrarily be assigned to a $DQ\alpha_1\beta_1$ dimer. The products of DP genes are detected by the response of T lymphocytes in the primed lymphocyte typing (PLT) assay (Sheehy et al. 1975) on the basis of which the DP polymorphism was defined (Shaw et al. 1980). Our reference in this paper to DR β1 or β2 genes and DR β1 or β2 proteins is not meant to relate a given gene to a given protein product.

similar levels of ignorance exist for DQ and DP.

RECOGNITION OF CLASS II PRODUCTS BY T LYMPHOCYTES

Specificities recognized by T lymphocytes are most commonly defined with homozygous typing cells (HTC) or primed lymphocyte typing (PLT) reagents; Specificities defined with HTCs are referred to as Dw (they are still ill-defined and thus referred to as "workshop" specificities designated with a "w"); the term LD is used for determinants recognized by T lymphocytes. An LD epitope is associated with a single molecular product; a Dw specificity is defined by the composite response of T lymphocytes to LD epitopes present on all products of HLA-D and thus represents a "haplotype designation."

Can the LD determinants (epitopes) recognized by T lymphocytes be defined serologically? This is a difficult, if not impossible, question to answer given present knowledge and techniques; nor, at least in the opinion of some, is it the most important question. Given currently available reagents, the determinants most frequently recognized by T cells are usually different from the determinants most readily recognized serologically. If it seems surprising that an epitope is not recognized by both T and B cells, one must remember that certain allogeneic differences recognized strongly by T lymphocytes (such as certain H-2K locus mutants (Bach et al. 1972, Widmer et al. 1973) have been extremely difficult to define serologically, despite very extensive efforts to do so.

The Homozygous Typing Cell (HTC) Test

Definition of the HLA-Dw1 through Dw19 specificities is based on proliferative responses of T lymphocytes in a primary mixed leukocyte culture (MLC) to stimulating cells that are putatively homozygous (HTC), at least for

the DR and DQ products. In some cases the HTCs are autozygous, i.e. the donors are offspring of consanguineous marriage, in which case the cells are almost always homozygous for all HLA-region products; in other cases, HTCs are phenotypically homozygous (allozygous) for DR and DQ but can be heterozygous for DP. Allozygous HTC may also not be identical in the two haplotypes for the DR and DQ products.

HTCs are chosen as typing reagents if i) the cells do not stimulate a significant response in the appropriate combinations within the family from which they were derived, ii) they do not stimulate (or are weak in stimulating) cells of other HTCs used to "define" the same Dw specificity, and iii) they can be used successfully to "type" an unrelated panel, i.e. to distinguish between unrelated individuals whose cells respond positively in MLC to the HTC and those who show no reponse or a weak reponse, i.e. carry the specificity defined by the HTC.

With some HTCs, responding cells of the panel form a biphasic reponse; those responding weakly are assigned the Dw specificity of the HTC, while those responding strongly are not. More often, however, there is no clear biphasic response and thus an arbitrary "cut-off" is chosen. It must be emphasized that this arbitrary threshold may represent as much as 30% of the T cell proliferative response, by those same responding cells, to antigenic differences associated with a full HLA-D disparity. Assignment of Dw specificities, therefore, frequently not only does not guarantee HLA-D region (including DP) identity between responding and stimulating cells but leaves a large likelihood of possible non-identity.

HTC testing has been most useful in defining the series of Dw specificities which represent clusters of antigenic determinants associated with the various class II products. (Class I antigens can, when disparate, also

stimulate T lymphocytes to proliferate, albeit weakly). The response to an HTC represents the aggregate reactions of clones that can recognize determinants associated chiefly with DR, DQ and DP. It depends not only on the number of determinants carried by the HTC which the responding cell does not have but also on the "strength" of those determinants, i.e. the frequency of clones that respond to those disparate determinants and the extent of proliferation by cells of those clones.

The ability to define Dw specificities with HTCs, despite the ability of all HLA products to stimulate T cell proliferation, rests to a great extent on three factors. First, the DR product(s) may stimulate most of the proliferating cells in an MLC (Bach et al. 1983) although there is only a little evidence for this. Second, loose criteria, i.e. a 40% to 50% relative response, have been used to designate a typing response; more stringent criteria, such as a 10% to 20% relative response, would result in fewer "typing responses." Third, sufficient linkage disequilibrium holds together on given haplotypes certain alleles encoding DR, DQ and other specificities; this linkage disequilibrium in turn leads to sufficient gene, and thus antigen, sharing between the HTCs and the cells of individuals being tested to provide typing responses. To the extent that HLA alleles, such as those of DP, are not in linkage disequilibrium with the DR-DQ combination of a given haplotype, stimulation of proliferation by products of those DP genes creates "noise," which makes more difficult the assignment of a Dw specificity. There is ample evidence that products are held together in linkage disequilibrium with different products in different populations. For instance, DR2 is found with DQw1 in the North American Caucasian population but with DQw3 in Southern American Waru Indians. Related but different Dw specificities may therefore be disclosed in different populations by a single HTC.

Polymorphism Within Serologically Defined DR and DQ (Ia) Specificities

There is polymorphism within single serologically defined (Ia) specificities of DR and DQ. Two major lines of evidence have led to this conclusion. First, individuals expressing a given DR Ia specificity, with the associated DQ Ia specificity (such as DR4-DQw3), can be divided into several groups based on assignment of Dw specificities. Second, protein studies of the β chains of DR and DQ have demonstrated a polymorphism in isoelectric focusing that correlates with the Dw types; this finding, mentioned in the introduction, will not be discussed extensively in this paper although it represents an important link in the overall story.

Certain relationships exist between the class II serologically defined (Ia) and T lymphocyte defined (Dw/LD) specificities. One speaks of one specificity as being "supertypic" to another (the latter being called "subtypic" to the first) based on population studies. For instance, DQw1 is generally supertypic to DR1, DR2 and DRw6; individuals positive for DQw1 can be divided into those that are positive for DR1, DR2 etc. Any individual typing positively for DR1, DR2 or DRw6 is also likely to carry the specificity DQw1. The DQ Ia and DRw52 and w53 specificities are supertypic to the DR1-DRw14 specificities; in addition, certain of the DR1-DRw14 specificities are supertypic to the various Dw specificities. The relationships of these specificities, including Dw, in terms of their being frequently encoded by a haplotype in the Caucasian population are given in Table 1.

The Dw specificities defined with HTCs were first thought by some to be the equivalent of the serologically defined DR antigens but there is now much evidence that one serologically defined DR specificity can be associated with several Dw subtypes as defined with HTC or PLT reagents. Although the Dw subtypes are usually referred to as being related to a given DR specificity, they

also relate to the supertypic DQ Ia specificities. These subtypes, given Dw designations, represent the aggregate effect of LD stimulating determinants associated with all of the HLA products. Thus, DQ and DR product-associated LD determinants contribute to the definition of the Dw specificity. One can appropriately speak of DR4-DQw3 or DR2-DQw1 Dw subtypes in the Caucasian population in which DR4 is generally in linkage disequilibrium with DQw3 and DR2 with DQw1.

At least four different Dw specificities can be defined within DR4-DQw3: Dw4, Dw10, Dw13 (formerly DB3) and Dw14 (formerly LD40); Dw15 (formerly DYT) is a subtype of DR4 but is not associated with DQw3. In addition, one of these, Dw13, can be further split using HTC, into KT2+ and KT2- cells (Reinsmoen and Bach 1982). Some DR4+ or DR2+ individuals carry "blank" (undefined) Dw specificities. This is shown graphically in Fig. 1. Similarly, DR2 can be split into three Dw subtypes; Dw2, Dw12 and a cluster of specificities including MN2 and LD-5a (Fig. 2). We have studied this DR2-associated cluster, LD-5a/MN2 in detail (Reinsmoen et al. 1984). The subtypes of DR2 and DR4 are listed in Table 2.

Primed Lymphocyte (LD) Typing (PLT)

The PLT test is an alternative method of defining LD determinants. HTCs are difficult to find and, as discussed above, carry LD determinants associated with class I and class II products that stimulate a proliferative response. In the PLT test there is selective activation of T lymphocytes to determinants associated with single or multiple class I or class II products. In order to develop a PLT reagent, responding and stimulating cells are chosen which differ for various class I and/or class II products. The cells are incubated in a primary MLC for a period of ten days, providing time for the responding cells to

revert to non-dividing, but now "primed", cells. These make an accelerated, and very strong, secondary response to the determinants recognized in the priming combination. When third party cells carry the LD specificities recognized in the sensitizing MLC, they will stimulate a proliferative reponse similar to that evoked by cells of the original sensitizing cell donor; third party cells not carrying those specificities will not evoke such a reponse.

As illustrated in Fig. 3, PLT reagents can be used to define determinants closely associated with HLA-Dw as defined by HTCs. These reagents are especially useful in defining new HLA-D specificities for which an HTC has not been defined. MN2 is a new HLA-D specificity associated with DR2 which has been defined by PLT not HTC reagents.

The cloning of PLT reactive cells has refined the PLT test. Clones presumably define single determinants; cloned reagents, therefore, can define determinants associated with a single product of the class II region. The alleles of DP, for instance, have to date only been defined with PLT reagents. Whereas it is extremely difficult to derive bulk PLT reagents to define the DP antigens, cloning of anti-DP reagents (generated in DR/DQ "identical" combinations) allows the ready preparation of PLT cells defining the DPw1-DPw3 and DPw5 and DPw6 antigens. Shown in Fig. 4 are results obtained with "cloned" PLT reagents defining the DPw2 and DPw4 specificities. Most "clones" primed to DPw2 are restimulated specifically by DPw2. The fact that DPw4, for instance, is usually "split" by using cloned reagents (Pawelec et al. 1982; Reinsmoen and Bach 1983) attests further to the power of PLT cell cloning. By choosing appropriate donors for the responding and stimulating cells for the generation of a PLT reagent, it is thus possible to obtain PLT reagents which correspond very closely to the Dw specificities defined with HTCs or to define individual LD determinants that make up a part of a Dw specificity.

Studies With "Cloned" T Cells

Although there is some evidence that DR and DQ products can stimulate T lymphocytes, it is not clear whether there are determinants associated with both these products that contribute to the Dw haplotype assignment, i.e. have a population distribution corresponding to the subtypic Dw specificity. We have thus used cloned T cells and panel studies of phenotypically well-characterized cells as well as monoclonal antibody blocking studies, to show that determinants associated with the DR as well as the DQ product appear to contribute to the definition of a Dw specificity (Reinsmoen and Bach 1985). Cloned T cells respond to what are probably two separate DR αβ dimers, although there is at present no evidence for a further subtype polymorphism of one of these dimers.

Utilizing cells from unrelated donors phenotypically identical for HLA-A and -B, two bulk primed reagents were generated: one against DR4, DRw53, Dw4, DQw3 disparities and the other against DR2, Dw2, DQw1 disparities. The bulk primed reagents were "cloned" by single cell deposition using the fluorescence activated cell sorter (FACS IV - Becton Dickinson) or by limiting dilution of one cell per three cells. The resulting "clonal" cell populations were expanded in IL-2 containing medium in the presence of x-irradiated feeder cells (lymphoblastoid cell line (LCL) of the original sensitizing cell). After three weeks' expansion, the clones were tested for proliferation in the PLT assay using a panel of well-characterized peripheral blood lymphocytes (PBL) stimulator cells, and for lytic reactivity in a micro CML assay using a panel of LCL target cells.

Panel Studies and Monoclonal Antibody Inhibition Studies of Cytolytic Clones

Table III illustrates the combined results of three CML testings using anti-DR4, -DRw53, -DQw3, and -Dw4 cells. The disparate priming specificities of the

original stimulator cell that are shared by the designated LCL targets are underscored as well as % cytotoxicity values of 10% or greater. The target cell panel included cells which typed for the five HLA-Dw specificities associated with DR4 (Dw4, Dw10, Dw13, Dw14, and Dw15) as well as DQw3 positive, DR4 negative cells and DQw3 negative, DR4 negative cells. All clones lysed the Dw4 positive targets including S (the original stimulator cell). Some clones (15, 33) lysed only the Dw4 target cells; other clones (67, 21, 56, 48) also lysed target cells which typed for other DR4 associated Dw specificities. In addition, certain clones (67, 56, 48, 21) lysed targets which did not type for DR4, Dw4, or DQw3 (targets 13, 15, 17, 18, 22).

Of 10 clones tested in mAb inhibition assays, the cytolytic activity of 9 was inhibited by the DR monomorphic mAb, L243 and Hu-4 (representative results illustrated in Table IV). The anti-DQ mAb, Tu22, also appeared to inhibit cytolysis in some cases by some of the clones; this mAb has been reported to have anti-DR activity (Pawelec et al. 1982). The cytolytic activity of clone 21 is not blocked by the anti-DR or -DQ mAb at the concentrations shown but is completely blocked (104% inhibition) at a 1:20 dilution of L243 in addition to being significantly inhibited by an anti-DRw53 mAb, PL3. This clone lyses 8 of 8 HLA-DRw53 positive target cells tested as well as 4 targets presumed to be DRw53 positive based on DR and DQ phenotyping (14 to 51% cytotoxicity), but does not lyse 7 DRw53 negative targets or 3 targets presumed to be DRw53 negative based on DR and DQ phenotyping (Table III) (-20 to 4% cytotoxicity). An intermediate lysis of 9% cytotoxicity was observed with the Dw15 HTC target which does not type as DRw53 with local antisera but does type as DRw53 positive with 9th International Histocompatibility Workshop antisera.

Clones were generated against DR2, Dw2, DQw1 disparate specificities using

unrelated cells which typed identically for HLA-A and -B and shared the DR3, Dw3, DQw2 and DPw4 specificities. Clones were obtained by single cell deposition (FACS IV) or limiting dilution of one cell per three wells. A total of 123 clones were tested in PLT and CML assays. Table V illustrates the combined results of three CML testings. All clones tested lysed the Dw2 targets tested. In addition some clones (1-12, 3-84) lysed the Dw12 target. Other clones (3-29, 3-27, 1-109) lysed additional DR2 positive targets. Clone 1-17 lysed the Dw2 positive targets as well as the DR2 negative, DQw1 positive targets tested. Clones 3-27 and 1-109 also lysed some DR2 negative, DQw1 negative targets.

Table VI demonstrated the patterns of target cell lysis by the 3 DR directed and 3 DQ directed (based on blocking with mAb (Reinsmoen and Bach 1985)) clones. Clone 3-19 lysed only Dw2 positive targets while clone 3-29 lysed all DR2 positive targets and clone 3-27 lysed some additional DR2 negative targets. Two of the DQ directed clones (3-89 and 3-17) lysed only Dw2 positive targets while clone 1-17 also lysed two additional DQw1 positive cells (targets 7 and 8).

Our results using cloned T cells are best discussed in the context of the working model that the extensive polymorphism for DR (DR1 through DRw14) is associated with an $\alpha\beta_1$ dimer and DRw52 and DRw53 with $\alpha\beta_2$. We would suggest that much, or perhaps essentially all, of the polymorphism seen by clones blocked with L243 and Hu4 and within DR4 or DR2 may be also associated with $\alpha\beta_1$ for both DR4 and DR2. Clones directed as both DR and DQ recognize determinants associated with the subtype polymorphism. This is in concert with findings of DR and DQβ chain polymorphism correlating with Dw subtypes.

Although we have only one clone putatively reactive with a determinant associated with DRw53 for DR4 haplotypes (blocked by PL3), this clone does not detect a polymorphism subtypic to the DR specificity of the $\alpha\beta_2$ dimer

(DRw53 and the presumed "DRw51"). There is increasing, albeit limited, evidence supporting the concept that the DRβ_2 gene/protein is relatively conserved on haplotypes carrying a single serologically defined specificity such as DR4 or DR2. First, Suzuki et al. (1984), Nishimura et al. (1985), and Knowles et al. (1985) have found one DR β spot in IEF that is relatively constant within the subtypes of DR2 and DR4, respectively; in the terminology we are using, this would be the DRβ_2 protein. Second, the clone (21) described in the present study as reactive with a potential DR $\alpha\beta_2$ dimer both react with essentially all the DR4 positive cells. Lastly, Cairns et al. (1985) in our laboratory have recently sequenced a gene from a DR4-Dw14 HTC, that is identical in sequence, with the exception of a single base pair substitution leading to a conservative amino acid change (lysine to arginine) at position 71, with a sequence obtained by E. Long from a cell carrying a DR4/Dw4 haplotype (or at least components of Dw4); such a sequence might code for DRw53. Thus, it may be that the more extensive T lymphocyte recognized polymorphism is associated primarily with DR$\alpha\beta_1$ and DQ and that DR$\alpha\beta_2$ is relative conserved.

THE DP POLYMORPHISM

The DP polymorphism has not been discussed in this chapter given its separate review in this volume (Sanchez-Perez and Shaw, this volume). Some comments regarding DP, especially as it may relate to DR and DQ would seem in order, however. First, reagents to define the DPw1-DPw6 segregant series may be more easily preparable by the "cloning" approach that we have discussed in the past (Bach and Reinsmoen 1982). Second, the protein polymorphism associated with DP could be studied by us following the recognition that the monoclonal antibody

B7/21 (Linner and Bach 1984) originally described by Royston et al. (1981), as an anti-DR mAb, in fact reacted with the DP dimer. This mAb was referred to as FA, with the approval of Trowbridge (Watson et al., 1983), to avoid confusion between LCL 721, with which most of the work was done to define this mAb as anti-DP, and the mAb. Third, it has been possible to show formally that FA most probably reacts with the αß dimer that expresses the DP determinant in that anti-FA blocks "clones" that react with the sensitizing DPw specificity (Ohta et al. 1985). Fourth, there is some added complexity of determinants recognized by cloned T lymphocytes which may, or may not, be related to the DP region (as defined with LCL mutants that have lost expression of all class I and of DR and DQ of a given haplotype, but still express DPw2 of that haplotype. Such mutants still express a determinant (called ND1) associated with that same haplotype that is different from DPw2 in its population distribution and based on the finding that ND1 reactive T cells are blocked with the mAb, Tu39, which is known to react with DP as well as other class II products (Ohta et al. 1985). Whether determinants recognized by this clone are related to the DP dimer as we currently think of it, or to a new αß dimer not previously described has yet to be determined.

We have suggested that the DP segregant series may be analogous in evolutionary terms to the LD subtype polymorphism related to DR of a single serologically defined specificity such as DR4. This suggestion is based primarily on two findings. First, restriction fragment length polymorphism (RFLP) patterns detected by Southern blotting is associated with different DR-DQ specificities are easily shown. It appears more difficult to define RFLP patterns associated with the various Dw/LD subtypes of a given serologically defined DR Ia specificity (Nicklas et al. 1985; Segall et al. 1985) as is also true for DP (Gorski et al. 1984). We have suggested that both the subtype polymorphisms of individual DR serologically defined specificities and the DP

polymorphism may be of more recent evolutionary origin (Bach 1985; Nicklas et al. 1985). Second, there is, to date, no convincing evidence that the several LD specificities of the polymorphism of DP can be reproducibly defined serologically, although some DPw correlated sera have been found, a situation similar to that which obtained for the DR4 and DR2 Dw subtypes.

DISCUSSION

The use of techniques that allow definition of determinants recognized by T lymphocytes and associated with HLA class II molecules has, thus, allowed definition of further polymorphisms not recognized with currently available/characterized serological reagents. Given the intimate relationship thought to exist between T lymphocyte recognition and major histocompatibility complex encoded molecules, definition of this LD (T lymphocyte defined), polymorphism may be of the greatest import.

There are several areas of interest in which Dw subtypes and serologically defined DR/DQ specificities have been compared for their respective roles. Two areas, which may well be interrelated, involve (i) studies of HLA class II restriction of nominal antigen for T cell recognition and (ii) HLA antigen association with insulin-dependent diabetes (IDD). We have recently performed/participated in a number of studies in which the class II restricting specificity for several different nominal antigens has been investigated vis-a-vis the serologically defined DR/DQ Ia specificities and the Dw specificities. Subtypes of DR4 and DR2 have been studied for these investigations. In most cases, the restricting specificity was very highly associated with, or identical to, a Dw subtype (Quigstad et al. 1984; Reinsmoen et al. 1984) rather than being associated directly with the serologically defined specificities. (In one case involving a DR2-Dw2 responding cell donor, the bulk reagents recognized the herpes virus in the context of both Dw2 and Dw12

but not in the context of DR2-MN2 or non-DR2 cells (Linner et al. 1985)). Interestingly, in this immunogenetic situation, we have found several alloreactive clones that were sensitized to a DR2-Dw2 haplotype, but that recognized a determinant apparently shared by DR2-Dw2 and DR2-Dw12 target cells. It would thus seem that the T lymphocyte recognized allo-LD specificities may directly serve, or act as markers, for restricted recognition.

We have also performed a subtype analysis in patients with IDD expressing either DR4 or DR2. In the DR4+ IDD patients, we found a very significant increase in Dw4 as opposed to the frequency of Dw4 in DR4+ normal individuals. In the DR2+ IDD patients, we found a very significantly decreased frequency of Dw2 and increased frequency of MN2 in the IDD patients as opposed to the normals (Bach et al. 1985). Once again, thus, it would appear that Dw/LD subtype definition may be of import for what are biologically important phenomena relating to the MHC.

That LD polymorphism, recognized by T lymphocytes, correlates with a protein polymorphism detected in isoelectric focusing and may well, to a large extent, reflect the functional polymorphism of these MHC loci as it pertains to restricted recognition. It would seem that a correlation between careful definition of class II determinants recognized by T lymphocytes can now be correlated with specific sequences of class II genes encoding those determinants. The tools of molecular biology make the future in this area very exciting indeed.

REFERENCES

1. Albertini RJ, Bach FH (1969) Quantitative Assay of Antigenic Disparity at HL-A--The Major Histocompatibility Locus in Man. J Exp Med 128:639
2. Amos DB, Bach FH (1968) Phenotypic expressions of the major histocompatibility locus in man (HL-A): leukocyte antigens and mixed leukocyte culture reactivity. J Exp Med 128:623
3. Bach FH (1985) The HLA-D region. Immunol Today 6:89
4. Bach FH, Amos, DB (1967) Hu-1: major histocompatibility locus in man. Science 156:1506-1508
5. Bach FH, Hirschhorn K (1964) Lymphocyte interaction: a potential histocompatibility test in vitro. Science 143:813
6. Bach FH, Voynow NK (1966) One-way stimulation in mixed leucocyte cultures. Science 153:545
7. Bach FH, Albertini RJ, Amos DB, Ceppellini R, Mattius PL, Miggiano VC (1969) Transplant Proc 1:339
8. Bach FH, Widmer MB, Segall M, Bach ML, Klein J (1972) Genetic and immunological complexity of major histocompatibility regions. Science 176:1024
9. Bach FH, Reinsmoen NR (1982) Cloned cellular reagents to define antigens encoded between HLA-DR and glyoxylase. Hum Immunol 5:133
10. Bach FH, Inouye H, Hank JA, Alter BJ (1979) Human T lymphocyte clones reactive in primed lymphocyte typing and cytotoxicity. Nature 281:307
11. Bach FH, Reinsmoen NL, Segall M (1983) Definition of HLA antigens with cellular reactants. Transplant Proc XV:102
12. Bach FH, Rich S, Barbosa J, Segall M (1985) Insulin dependent diabetes--associated HLA-D region encoded determinants. Hum Immunol 12:59

13. Bain B, Vas MR, Lowenstein L (1964) Blood 23:108

14. Dupont B, Jersild C, Hansen GS, Nielsen S, Thomsen M, Svejgaard A (1973) Typing for MLC determinants by means of LD homozygous and LD-heterozygous test cells. Transplant Proc 5:1453

15. Fathman CG, Hengartner H Clones of alloreactive T cells. (1978) Nature 272:617

16. Gorski J, Rollin R, Long E, Mach B (1984) Molecular organization of the HLA-SB region of the human major histocompatibility complex evidence for two SB beta chain genes. Proc Natl Acad Sci USA 81:3934

17. Groner JP, Watson AJ, Bach FH (1983) Dw/LD-related molecular polymorphism of Dr4 β-chains. J Exp Med 157:1687

18. Goyert SM, Catti R, Silver J (1982) Peptide map comparisons of similar serologically defined HLA-DR antigens isolated from different lymphoblastoid cell lines. Hum Immunol 5:205

19. Hurley CK, Giles RC, Nunez G. DeMars R, Nadler L, Winchester R, Stastny P, Capra JD (1984) Molecular localization of human class II MT2 and MT3 determinant. J Exp Med 160:472

20. Jorgensen F, Lamm L, Kissmeyer-Nielsen F (1973) An approach to MLC typing. Tissue Antigens 4:323

21. Linner KM, Bach FH (1985) A non-DR, non-DC HLA class II product. In: ED Albert, MP Baur, WR Mayr (eds) Histocompatibility Testing 1984, Springer-Verlag, New York p538

22. Mempel W, Grosse-Wilde H, Albert E, Thierfelder S (1973) Atypical MLC reactions in HL-A typed related and unrelated pairs. Transplant Proc 5:401

23. Nepom BS, Nepom GT, Mickelson E, Antonelli P, Hansen JA (1983) Electrophoretic analysis of human HLA-DR antigens from HLA-DR4 homozygous cell lines: Correlation between β-chain diversity and HLA-D. Proc Natl Acad Sci USA 80:6962

24. Nicklas JA, Noreen HN, Segall M, Bach FH (1985) Southern analysis of DNA polymorphism between Dw subtypes of DR4. Hum Immunol (in press)

25. Nishimura Y, Tsukamoto K, Sone T, Hirayama K, Takenouchi T, et al (1985) Molecular and functional analysis of class II molecules characteristic to HLA-Dw2 and Dw12. In: E Albert (ed) Munksgaard Copenhagen, Histocompatibility Testing 1984 p 504

26. Pawelec G, Shaw S, Wernet P (1982) Analysis of the HLA-linked SB gene system with cloned and uncloned alloreactive T-cell lines. Immunogenetics 15:187

27. Pawelec G, Shaw S, Schneider M, Blaurech M, Frauer M, Brackertz D, Wernet P (1982) Population studies of the HLA-linked SB antigen and their relative importance in primary MLC-typing analysis of HLA-D homozygous typing cells and normal heterozygous populations. Hum Immunol 5:215

28. Pawelec GP, Shaw S, Ziegler A, Muller C, Wernet P (1982) Differential inhibition of HLA-D-or SB-directed secondary lymphoproliferative responses with monoclonal antibodies detecting human Ia-like determinants. J Immunol 129:1070

29. Qvigstad E, Thorsby E, Reinsmoen NL, Bach FH (1984) Close association between the Dw14 (LD40) subtype of HLA-DR4 and a restriction element for antigen specific T cell clones. (in press)

30. Reinsmoen NL, Bach FH (1983) HLA-D region complexity associated with HLA-DR, Dw and SB phenotypes. Transplant Proc 15(1):76

31. Reinsmoen NL, Bach FH (1983) HLA-D region complexity associated with HLA-DR, Dw and SB phenotypes. Transplant Proc 15(1):76

32. Reinsmoen NL, Layrisse Z, Betuel H, Bach FH (1984) A study of HLA-DR2 associated HLA-Dw/LD specificities. Hum Immunol 11:105

33. Royston I, Omary MB, Trowbridge IS (1981) Monoclonal antibodies to human T-cell antigen and Ia-like antigen in the characterization of DR4 β-chains. Transplant Proc XIII:761

34. Shaw S, Jonhson AH, Shearer GM (1980) Evidence for a new segregant series of B cell antigen that are encoded in the HLA-D region and that stimulate secondary allogeneic proliferative and cytotoxic responses. J Exp Med 152:565

35. Sheehy MJ, Sondel PM, Bach ML, Wank R, Bach FH (1975) HL-A LD (lymphocyte defined) typing: A rapid assay with primed lymphocytes. Science 188:1308

36. Suzuki M, Yabe T, Satake M, Jeiji T, Hamaguchi H (1984) Two-dimensional gel electrophorite analysis of the MT3 and DR4 molecule from the different D-typed cells. J Exp Med 160:751

37. Van den Tweel JG, Blusse van Oud Alblas A, Keuning JJ, Goulmy E, Termijtelen A, Bach ML, Van Rood JJ (1973) Typing for MLC (LD) 1. Lymphocytes from cousin marriage offspring as typing cells. Transplant Proc 5:1535

38. van Leeuwen A, Schuit HRE, van Rood JJ (1973) Typing for MLC (LD) II. The selection of nonstimulator cells by MLC inhibition tests using DS-identical stimulator cells (MISIS) and fluorescence antibody studies. Transplant Proc 5:1539

39. Watson AJ, DeMars R, Trowbridge IS, Bach FH (1983) Detection of a novel human class II HLA antigen. Nature 304:358

40. Widmer MB, Alter BJ, Bach FH, Bach ML, Bailey DW (1973) Lymphocyte reactivity to serologically undetected components of the major histocompatibility complex. Nature New Biol 242:239

41. Yunis EJ, Amos DB (1971) Three closely linked genetic systems relevant to transplantation. Proc Natl Acad Sci USA 68:3031-3035

Table I Established HLA-D Region Specificities Defined by the 9th International Histocompatibility Workshop 1984

HLA-DR	Specificities	HLA-Dw Association	HLA-DQ Association
DR1	-	Dw1	DQw1
DR2	-	Dw2	DQw1
	-	Dw12	DQw1
DR3	DRw52	Dw3	DQw2
DR4	DRw53	Dw4,10,13,14	DQw3
		Dw15	DQ blank
DR5	DRw52	Dw5	DQw3
DRw11	DRw52	-	DQw3
DRw12	DRw52	-	DQw3
DR6			
DRw13	DRw52	Dw18,19	DQw1
DRw14	DRw52	Dw9	DQw1
DRw14	DRw53	Dw16	DQw3
DR7	DRw53	Dw7,17	DQw2
	DRw53	Dw11	DQw3
DRw8	DRw52	Dw8	DQ blank
DRw9	DRw53	-	DQw3
DRw10	-	-	DQw1

Table II. HLA-DR2 and DR4 Associated Dw Specificities
University of Minnesota

	#	% of DR2 or DR4 haplotypes	Ag freq.	Gene freq.
Dw2	120	78%	0.134	0.069
Dw12	7	5%	0.008	0.004
MN2	10	6%	0.011	0.006
Dw blank	17	11%		
Dw4	84	43%	0.094	0.048
Dw14	41	21%	0.046	0.023
Dw10	18	9%	0.020	0.010
Dw13	15	8%	0.017	0.009
Dw15	4	2%	0.004	0.002
Dw blank	32	16%		

total # HLA-Dw typed = 896

TABLE III Cytolytic Clones - Anti DR/Dw4 Priming*

Targets

	S1	1	2	3	4	5	6	7	8	9	10
DR	3,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4
DRw	53	(53)	(53)	53	(53)	(53)	53	53	-	53	53
Dw	3,4	4,4	4,4	13,4	13,13	13,13	14,14	14,14	15,15	10,10	10,10
DQ	3	3	3	(3)	(3)	3	3	3	-	3	3
DP	1	4	3,4	NT	NT	5	2,3	3,6	5	NT	4
clone #											
15	18	34	25	15	3	1	-2	-19	2	-2	3
33	43	62	46	34	1	8	8	-10	2	-4	1
67	35	59	44	46	-1	43	-1	-19	3	1	68
21	14	46	24	22	17	14	26	17	9	17	71
56	31	64	35	32	62	24	55	NT	25	-4	3
48	14	46	25	27	34	5	62	64	19	10	3

Targets

	11	12	13	14	15	16	17	18	19	20	21	22
DR	5,5	5,5	3,3	2,2	1,9	2,6	2,2	1,1	2,3	3,3	6,6	7,7
	52	NT	52	NT	53	52	-	-	52	NT	52	53
Dw	5,5	5,5	3,3	2,2	1,BSK	6,MN2	2,2	1,1	2,3	3,3	6,6	7,7
DQ	3	NT	2	NT	1	1	1	1	1	NT	1	2
DP	4	4	3,4	4,5	NT	NT	2,5	3,4	4,6	1,3	2,4	4
clone #												
15	-5	-4	6	1	3	-4	-2	-5	-5	-18	-9	-7
33	-6	-7	2	-2	4	-6	-6	3	-4	-2	-3	-4
67	2	6	10	NT	NT	-2	16	2	7	-4	-2	-1
21	-3	-5	-5	4	51	-2	-4	-2	2	-20	-7	29
56	-4	-5	12	8	11	-4	-4	-5	-7	-20	-8	-10
48	-7	-6	4	9	49	-5	-6	55	4	-12	-8	-5

*clones 56, 63, and 46 also demonstrate proliferative reactivity

Specificities indicated within parenthesis have not been tested but are as indicated based on the phenotyping data.

TABLE IV Dissection of Determinants Associated with Different DR Dimers Anti-DR4/Dw4/DQw3 Priming Combination

	Anti-DR mAb						Anti-DRw53 mAb			Anti-DQ mAb		
	L243			Hu4			PL3			Tu22		
Clones	80**	400	4000	80	400	4000	80	400	4000	80	400	4000
15	113*	104	60	121	98	54		NT		14	25	31
37	121	129	121	111	79	57		NT		43	32	67
48	116	106	66	91	81	6	29	-29	-43	-3	9	3
67	111	89	38	117	109	57	24	17	24	-13	-11	2
63	97	32	38	145	104	31	-25	-150	0	38	45	62
21	7	8	14	20	2	10	101	104	76	14	5	29

* results expressed as % inhibition
value > 50% are underscored
NT = not tested

** reciprocal mAb dilutions

TABLE V Cytolytic Clones - Anti DR2/Dw2 Priming*

	Targets																	
	S1	S2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DR	2,3	2,3	2,2	2,2	2,2	2,2	2,2	2,2	6,6	1,1	4,4	4,4	4,4	4,4	3,3	3,3	7,7	5,5
Dw	2,3	2,3	2,2	2,2	2,2	12,12	FJO	LD-5a	6,6	1,1	4,4	10,10	14,14	13,13	3,3	3,3	7,7	5,5
DQ	1,2	1,2	1	1	NT	1	1	3	1	1	3	3	3	NT	NT	2	2	3
DP	4,6	4	4,5	2,5	NT	NT	NT	NT	2,4	3,4	6,2	4	6,3	NT	1,3	3,4	4	4
clone #																		
3-89	19.1	38.3	5.2	23.3	41.4	7.8	1.3	-2.8	-7.8	-7.5	2.4	-.1	-.7	.1	6.4	-16.5	-1.2	.2
3-17	23.8	25,5	19.4	28.7	29.0	3.5	1.9	-2.8	-3.6	-8.0	3.6	-.6	-.9	-.7	4.2	-19.1	1.7	2.9
1-12	27.7	43.5	35.1	54.0	40.4	19.8	-.5	-.6	-5.5	2.0	-.9	-1.4	-2.1	-2.7	-.6	-17.3	-3.5	-.7
3-84	11.1	21.5	13.8	43.5	40.4	30.4	4.1	-2.6	-6.9	-4.9	3.3	-.2	-.5	-1.5	1.4	-16.3	-1.2	4.5
3-29	26.8	27.0	21.6	40.9	30.7	29.2	46.6	42.8	-8.3	-7.1	8.1	-.7	-2.8	-4.2	12.5	-16.3	-2.0	-1.2
1-17	11.2	20.1	22.4	20.0	24.1	.5	4.7	-3.1	51.0	26.2	.7	-.7	-2.0	-2.9	-.6	-17.1	-1.3	-.6
3-27	27.2	44.9	17.6	53.1	49.8	35.9	17.7	-1.3	46.9	-4.8	4.4	51.0	-.2	-1.3	1.8	-15.3	2.0	3.9
1-109	14.6	56.7	33.6	55.0	44.0	8.8	16.1	44.8	12.6	-8.2	14.3	37.4	-3.0	-3.0	5.6	-18.2	22.9	4.3

*clone 3-19, 1-84, 3-91, 3-29, 3-27, 1-109 also demonstrated strong proliferative reactivity (>10,000 cpm) and clc
3-89, 3-17, and 1-17 demonstrated weaker proliferative reactivity

TABLE VI HLA-DR and DQ Determinants Comprising a "Dw Specificity"

		LCL target cells								
		S	1	2	3	4	5	6	7	8
HLA	DR	2,3	2,2	2,2	2,2	2,2	2,2	2,2	6,6	1,1
	Dw	2,3	2,2	2,2	2,2	12,12	PJO	LD-5a	6,6	1,1
	DQw	1,2	NT	1	NT	1	1	3	1	1
	DPw	4,6	4,5	2,5	NT	NT	NT	NT	2,4	3,4
Anti-DR Clones	3-19	15	14	40	55	4	1	-2	-2	-5
	3-29	27	22	41	31	29	47	43	-8	-7
	3-27	27	18	53	50	36	18	-1	47	-5
Anti-DQ Clones	3-89	19	5	23	41	8	1	-3	-8	-8
	3-17	24	19	29	29	4	2	-3	-4	-8
	1-17	11	22	29	24	1	5	-3	51	26

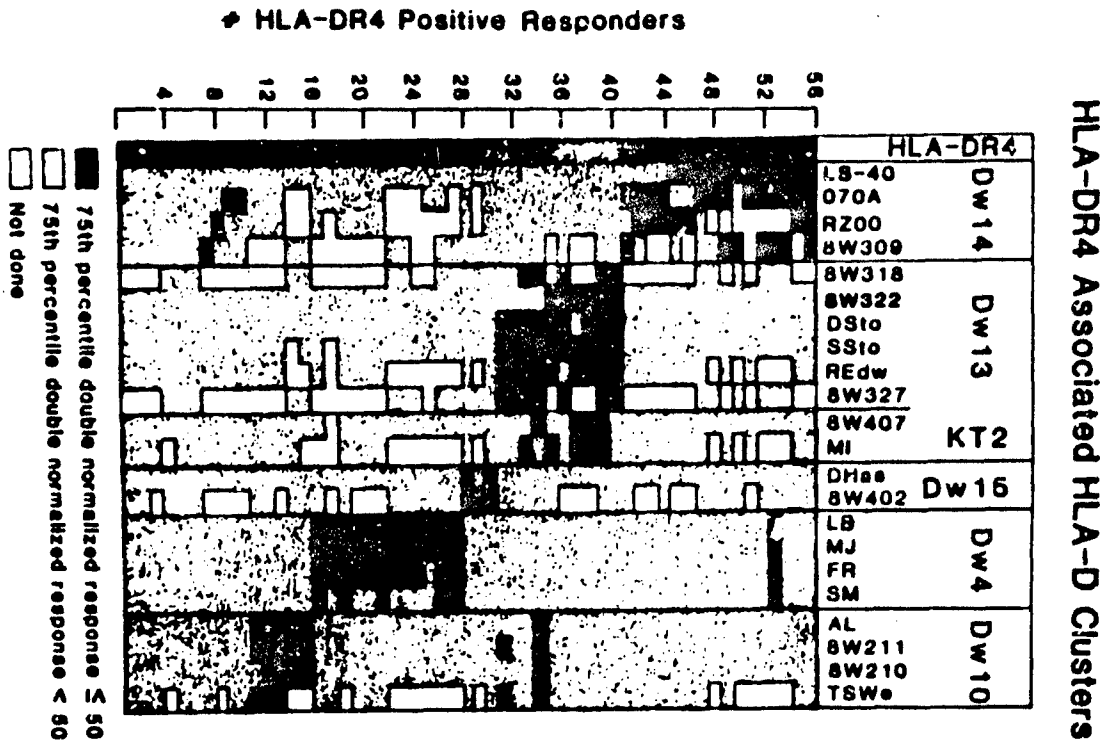
Legends

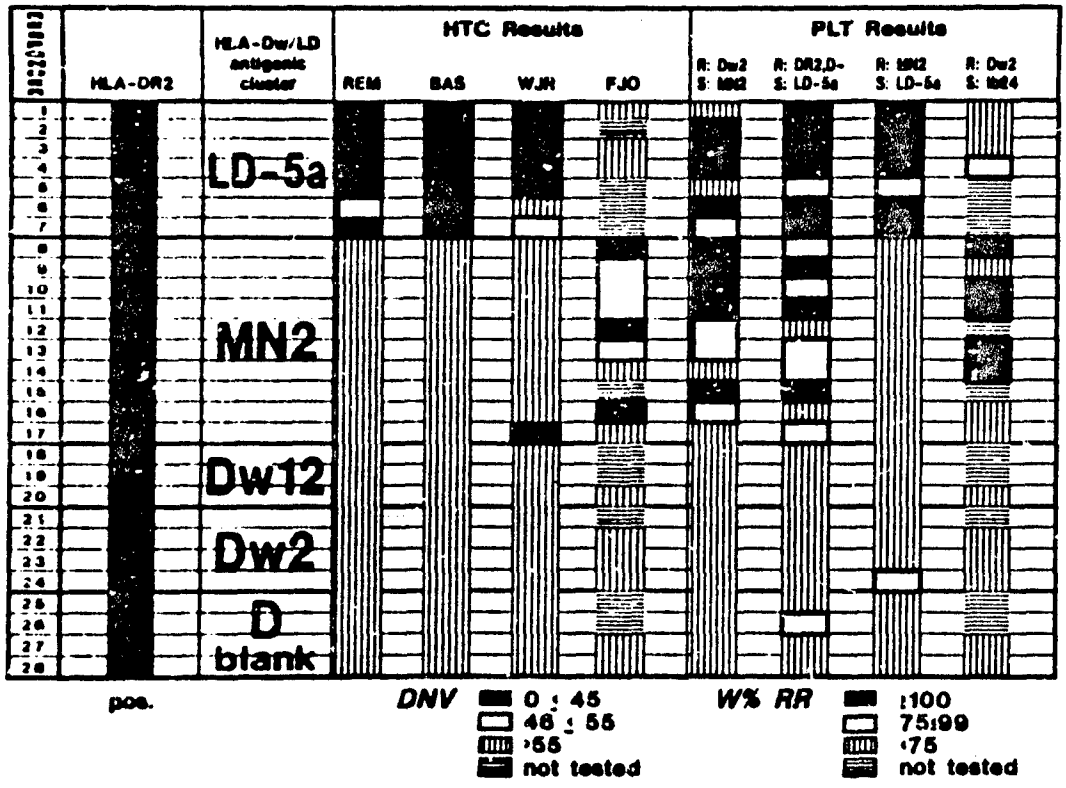
Fig. 1. Five HLA Dw Clusters Associated with HLA-DR4: 56 DR4+ cells were typed with 22 DR4+ HTCs defining the Dw clusters Dw14, Dw13 with the associated specificity KT2, Dw15, Dw4, and Dw10.

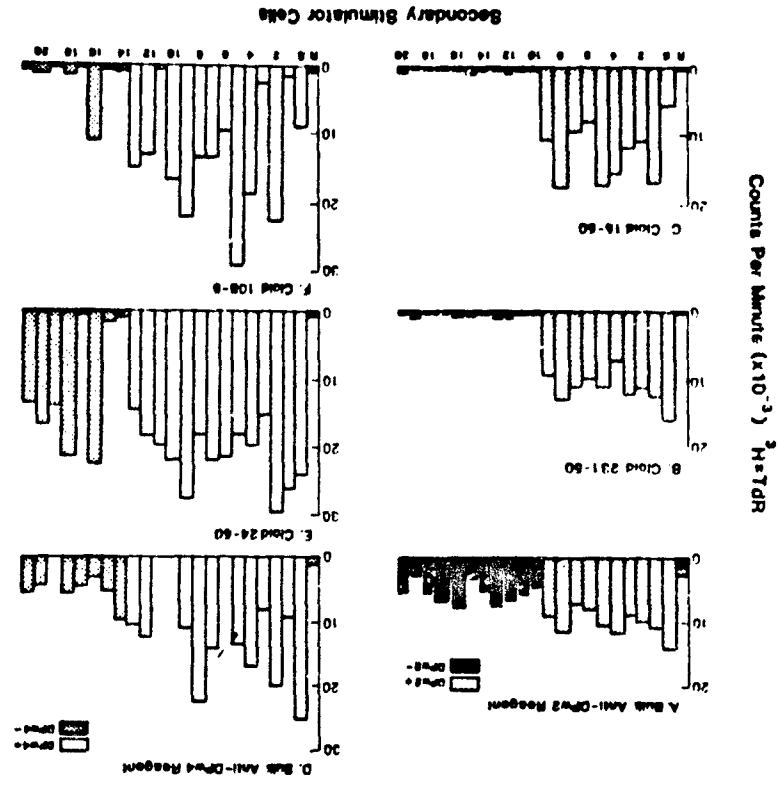
Fig. 2. HLA-DR2 Associated Dw clusters: 28 DR2+ cells classified as typing for Dw2, Dw12, MN2, LD-5a or blank were tested by the LD-5a HTCs, REM and BAS, as well as a related HTC WJR and a fourth DR2+ HTC, FJO. Only LD-5a+ responder cells gave typing responses to the LD-5a HTCs whereas some but not all MN2+ cell gave typing response to the FJO HTC. When cells were primed against either MN2+ or LD-5a+ haplotypes both LD-5a+ and MN2+ cells restimulated both these primed reagents suggesting shared stimulator determinants. By careful selection of the priming cell combinations used to generate additional PLT reagents, stimulatory determinants unique to the LD-5a or MN2 specificities could also be identified.

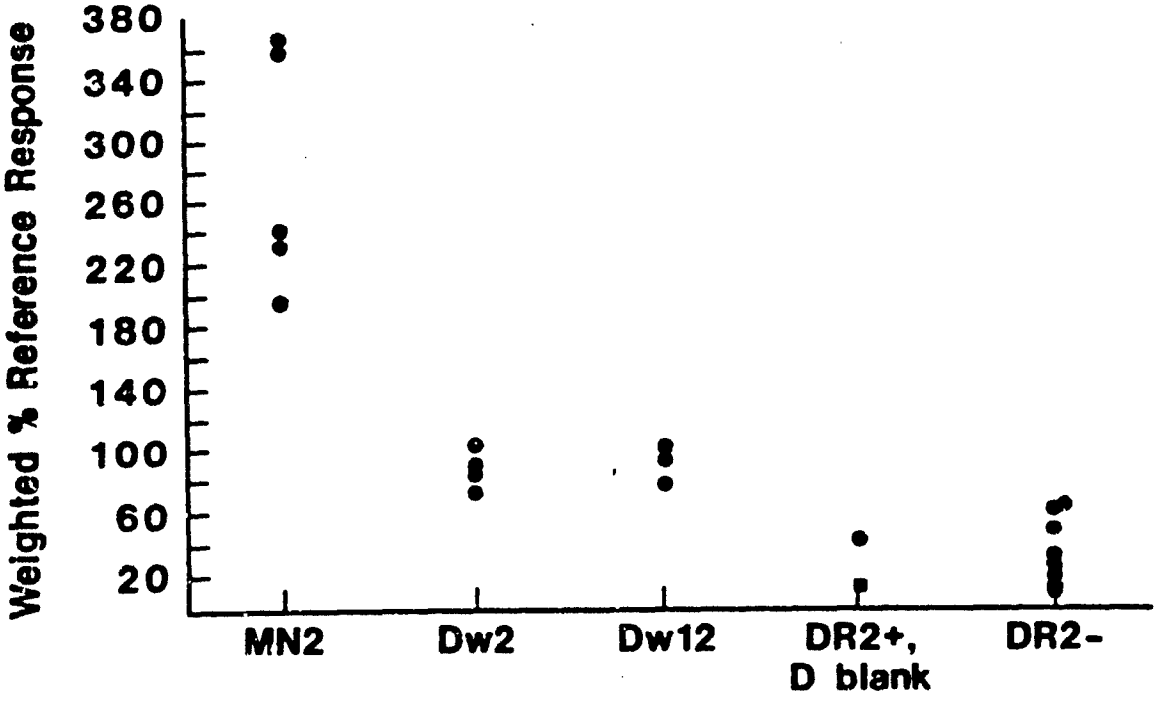
Fig. 3. Definition of the HLA-DR2 associated specificity MN2 as defined by PLT reagents: PLT reagent generated from responding cell HLA-A3,2; B44,15; DR1; Dw1 primed against stimulator cell HLA-A2, w32; B15, w39; DR1,2; Dw1, MN2 was restimulated by Dw2+, Dw12+, DR2+ Dw blank and DR2 negative cells.

Fig. 4. PLT results of two bulk reagents prepared against DPw2 (A) and DPw4 (D) and two cloids derived from each bulk reagent (B, C and E, F respectively) utilizing the following priming combinations: anti-DPw2: R = HLA-A2, 11; B7, w35; DR1,7; Dw1,7; DPw3,4; S = HLA-A2, 25; Bw39, w50; DR1,7; Dw1,7; DPw3,2; and anti DPw4; R = HLA-A2; B40; DR4,7; Dw4,7; DPw2; S = HLA-A1, w31; B17, 40; DR4,7; DW4,7; DPw2,4. Cloids refer to reagents generated from plating a limited number of cells (i.e. 5) per well and are thus probably not true clones.


HLA-DR4 Positive Responders
4 8 12 16 20 24 28 32 36 40 44 48 52 56
HLA-DR4
LS-40
070A
RZ00
8W309
Dw14
8W318
8W322
DSto
SSto
REdw
8W327
Dw13
8W407
MI
KT2
DHaa
8W402
Dw15
LB
MJ
FR
SM
Dw4
AL
8W211
8W210
TSWe
Dw10
75th percentile double normalized response ≥ 50
75th percentile double normalized response < 50
Not done
HLA-DR4 Associated HLA-D Clusters


HLA-DR2
HLA-Dw/LD antigenic cluster
HTC Results
REM
BAS
WJR
FJO
PLT Results
R: Dw2 S: MN2
R: DR2,D- S: LD-5a
R: MN2 S: LD-5a
R: Dw2 S: Dw4
LD-5a
MN2
Dw12
Dw2
D
blank
pos.
DNV
0 : 45
46 : 55
>55
not tested
W% RR
≥100
75:99
<75
not tested


Secondary Stimulator Cells
Counts Per Minute (x10⁻³) ³H-TdR


Weighted % Reference Response
380
340
300
260
220
180
140
100
60
20
MN2
Dw2
Dw12
DR2+,
D blank
DR2-